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Immunotherapy Targeting Leukemia Stem Cells

Maiko Matsushita*

Division of Clinical Physiology and Therapeutics, Keio University Faculty of Pharmacy, Tokyo, Japan
*Corresponding author: matsushita-mk@pha.keio.ac.jp

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Abstract Leukemia stem cells (LSCs) are considered to cause treatment failure and disease progression in leukemia patients. Although LSCs are rare, they are a quiescent population and so cannot be targeted by conventional therapies such as chemotherapy, targeted therapy, and radiotherapy. On the other hand, immunotherapy including hematopoietic stem cell transplantation could target LSCs regardless of cell cycle status. Identification of LSC-specific antigens is important for developing effective immunotherapies. Several antigens highly expressed in LSCs or on the surface of LSCs have been reported, and some of them have been found useful for eradication of LSCs. Cancer vaccines, adoptive T cell therapy, and antibody treatments to target these antigens are strategies expected to be used in the near future.

Keywords: *immunotherapy, leukemia stem cells, antigens*

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1. Introduction

In spite of advancements in treatment options, cancer recurrence is frequently observed. Human cancers consist of a heterogeneous population maintained by cancer stem cells (CSCs), which are considered to cause treatment failure and disease progression [1]. CSCs are a rare population of cancer cells that possess characteristics of normal stem cells—the potential to self-renew, differentiate, and proliferate [2,3,4]. The CSC model was first established in human acute myeloid leukemia (AML). Dick et al. identified CSCs in AML using clonal in vivo repopulation assays. They found that leukemic stem cells are enriched in the $Lineage^-/CD38^-/CD34^+$ compartment of $CD34^+$ AML cells [2].

LSCs are resistant to conventional therapies, such as chemotherapy and radiotherapy [5,6], which target proliferating cancer cells. Even targeted therapy is ineffective at eradicating LSCs, and remaining LSCs cause disease relapse [7]. For example, tyrosine kinase inhibitors (TKIs) such as imatinib have dramatically improved survival of chronic myelogenous leukemia (CML) patients; however, about half of the patients eventually relapse after cessation of the drug [8,9,10], suggesting that LSCs remain after TKI treatment. Although the precise mechanism of CML-LSC's resistance to TKIs is not fully understood, the existence of patients who do not relapse after cessation of TKI suggests the possibility that a patient's immune system could eradicate LSCs. Therefore, targeting LSCs by immunotherapy is an attractive strategy to develop a cure for leukemia patients.

In this review, potential therapeutic targets that could be utilized in various types of immunotherapy—including cancer vaccines, adoptive T cell therapy, and antibody treatments—will be discussed.

2. LSC-antigens for Cancer Vaccine or Adoptive T Cell Therapy

Hematopoietic stem cell transplantation (HSCT) is now considered as immunotherapy because donor-derived T lymphocytes included in the graft can attack residual leukemia cells [11,12]. This mechanism is defined "graft-versus leukemia (GVL) effect". Donor lymphocyte infusion (DLI) is also effective in leukemia patients who relapse after HSCT [13]. These observations indicate the potential of immunotherapy in leukemia treatment. In those settings, allo-antigens, such as major histocompatibility antigen (MHC) or minor histocompatibility antigens, could elicit immunity against donor-derived cells [14,15,16]. However, these antigens are also expressed in recipients' normal cells, causing graft-versus-host disease (GVHD) in multiple tissues, including skin, liver, and gastrointestinal tract [17]. In addition, some patients still relapse after the treatment, which suggests these treatments are not sufficient for eradication of LSCs. Therefore, immunotherapy targeting antigens specifically expressed in LSCs is needed.

The National Cancer Institute has reported a list of cancer antigens for immunotherapy, which includes stem cell expression of each candidate antigen [18]. Among them, Wilms' tumor gene 1 (*WT1*) is ranked first as a target antigen for immunotherapy. *WT1* is highly expressed in various cancer cells including leukemia cells. It has been reported that this gene is overexpressed in 93% of AML patients and 79% of ALL patients [19]. Moreover, its expression is correlated with poor prognosis in newly diagnosed AML patients [20], which suggests that this

gene is also expressed in LSCs. In fact, Ishikawa et al. showed that WT1 is expressed in cell cycle–quiescent primary AML LSCs at the endosteal region [21]. These findings provide rationale for the use of vaccination with WT1-derived peptides, protein, or adoptive T cell therapy with WT1-directed TCR gene-transduced T cells for immunotherapy for leukemia patients [22,23,24,25].

Gerber et al. detected expression of known tumor antigens in $CD34^+CD38^-ALDH^{high}$ cells from CML patients and found that not only WT1 but also PRAME (preferentially expressed antigen of melanoma) is highly expressed in CML LSCs. On the other hand, they showed that other antigens expressed in myeloid leukemia cells, such as Proteinase 3, SURVIVIN, and hTERT, were expressed in CML LSCs at low levels. Thus, they suggest that WT1 and PRAME might be promising targets for LSC-directed immunotherapy [26].

The *PRAME* gene is highly expressed in various cancer cells. With regard to hematological malignancies, 42% of patients showed high levels of PRAME expression [27]. Several epitopes have been reported, and anti-PRAME CTLs have been shown to eradicate leukemic cells without affecting normal hematopoietic progenitors [28,29].

Recent studies have revealed other genes or gene mutations in LSCs [2,30]; therefore, the epitope antigens derived from those abnormal genes would also be good target antigens for cancer vaccines or adoptive T cell therapies.

3. Antibodies Targeting LSCs

Since the development of emerging technologies of antibody production, immunotherapy using monoclonal antibodies against specific antigens expressed on cancer cells has become very successful [31,32], and may be applied to eradication of LSCs [33]. However, cell-surface antigens expressed on LSCs are often also expressed on normal hematopoietic stem cells. Among those common surface antigens, several CD antigens including CD25, CD26, CD44, CD96, and CD123 are relatively highly expressed in LSCs compared to non-LSCs.

Several reports have shown that CD25 is highly expressed in some CML LSCs [34]. Kobayashi et al. showed that $CD25^+$ $Fc\varepsilon RI\alpha^-Lin^-Sca\text{-}1^+C\text{-}Kit^+$ leukemia initiating cells (LICs) are more capable of reconstituting leukemia than $CD25^-$ LICs in a CML mouse model [35]. They also revealed that administration of anti-CD25 monoclonal antibodies prolonged survival of CML mice. However, from the standpoint of immunotherapy, systemic blockade of the CD25 molecule might be not favorable because CD25 is an α chain of the interleukin-2 (IL-2) receptor, which is also expressed in other normal immune cells.

Herrmann et al. reported that CD26 (dipeptidylpeptidase IV) is expressed in the LSC fraction of CML patients, although the molecule was not detected in normal HSCs [36]. They also showed that $CD26^+$ LSCs produced BCR/ABL-positive colonies, while $CD26^-$ LSCs did not, suggesting that CD26 is critical for early development of CML. Therefore, targeting CD26 would be an attractive way to eradicate LSCs.

CD96 belongs to the immunoglobulin superfamily and is strongly expressed in $CD34^+CD38^-$ AML cells, but not in the normal hematopoietic stem cell population [37]. In addition, $CD96^+$ LSCs have a high capacity for engraftment in mice. Thus, it may be possible to use this molecule for LSC-targeted antibody therapy.

It has also been reported that anti-CD123 antibodies can target AML LSCs and profoundly reduce AML LSC engraftment [38]. Moreover, anti-CD123 chimeric antigen receptors (CAR)-transduced T cells have been shown to be an attractive immunotherapeutic tool for AML treatment [39].

T cell immunoglobulin mucin (TIM)-3 is selectively expressed in LSCs of AML except acute promyelocytic leukemia. Kikushige et al. showed that mouse anti-human TIM-3 antibodies eradicated human AML cells engrafted in immunodeficient mice by complement-dependent and antibody-dependent cellular cytotoxic activities, without blocking engraftment of normal hematopoietic cells [40]. TIM-3 is also known as an immune-suppressing molecule, which enhances the importance of blocking this antigen [41].

It has been shown that a variant form of CD44, CD44-6v, is overexpressed on several CSCs including leukemic $CD34^+CD38^-$ cells [42]. CD44 is essential for homing of AML LSCs to hyaluronan, a glycosaminoglycan highly concentrated in bone marrow niches, to keep these cells in a primitive state. It has also been reported that elevated CD44-6v expression on AML cells is associated with poor prognosis in AML patients. Jin et al. reported that monoclonal antibodies against CD44 caused marked reduction of primary AML cells in immunodeficient mice [43]. These findings suggest that blockade of the interaction between LSCs and the niche is also an effective strategy to prevent proliferation of LSCs.

Recent proteomics approaches using a large panel of AML $CD34^+$ and normal bone marrow $CD34^+$ samples have revealed CD82, CD97, CD99, PTH2R, ESAM, MET, and ITGA6 as other surface antigens expressed on AML LSCs [44]. Further investigation would be necessary to clarify the importance of these antigens as therapeutic targets.

4. Future directions.

Although many antigens expressed in LSCs have been identified, strong immunity against these antigens should be evoked to treat leukemia patients in clinical settings.

Cancer vaccines, adoptive T cell therapy, and antibody treatments are major immunotherapy options for cancer treatments (Table 1). Cancer vaccine using antigenic peptides or antigen-expressing dendritic cells have been tried in clinical trials, however, their clinical benefits remain to be proven. Thus, additional methods to boost antigen-specific immunity should be added to these vaccine settings. Blocking of regulatory T cells might be one of such strategies.

Adoptive T cell therapy including antigen-specific TCR gene-modified T cells and CAR-T cells could evoke strong immunity against LSCs. The simple manufacturing system of gene-modified T cells for each patient should be established to apply these treatments for a large number of leukemia patients. Antibody treatments are now broadly

used in cancer treatment and development of clinical grade antibodies against each cell surface antigens of LSCs is expected. Antibody-drug-conjugate (ADC) is also useful for eradication of LSCs, since this type of antibody could deliver highly cytotoxic agents to LSCs.

Table 1. Immunotherapy targeting LSC-antigens

Type of immunotherapy	Potential target antigens
Vaccines	
Cancer peptide vaccines	WT1, PR-3, PRAME, etc
DC vaccines	WT1, PR-3, PRAME, etc
Adoptive T cell therapy	
TCR gene-modified T cells	WT1
CAR-T cells	CD25, CD26, CD44, CD96, CD123, TIM3, etc
Antibodies	CD25, CD26, CD44, CD96, CD123, TIM3, etc

Other promising strategy is a combination of immunotherapy and targeted therapy or chemotherapy.

Eradicating only LSCs by immunotherapy cannot sufficiently cure of leukemia, since LSCs are a quite small population of total leukemia cells.

In the case of CML, TKIs are the gold standard for reducing leukemia cells. However, several TKI cessation clinical trials show that they cannot completely eradicate LSCs in some patients. Thus, the strategy to conduct LSC-targeted immunotherapy after reduction of non-LSCs by TKIs would be useful. Alternatively, conducting those treatments simultaneously may also be effective, depending on the type of TKI used. Specifically, dasatinib is known to enhance patients' immunity against CML cells. It has been reported that lymphocytes with a large granular phenotype (LGLs) are observed in 30–60% of patients with CML or Ph-ALL treated with dasatinib [45]. LGLs are often NK cells or $CD8^+$ cytotoxic T cells that might be able to directly attack leukemia cells [46]. The combination of these immune-boosting drugs and immunotherapies targeting LSCs might be an ideal treatment.

It has also been reported that chemotherapeutic drugs synergize with immunotherapy through several mechanisms. For example, cyclophosphamide or fludarabine could promote the effect of immunotherapy by homeostatic proliferation of $CD8^+$ T cells [47]. Gemcitabine is known to sensitize tumor cells to CTL-mediated cytotoxicity and also eradicate myeloid–derived suppressor cells [48]. Therefore, combination of these chemotherapies with LSC-targeted immunotherapy is an attractive treatment option for leukemia patients.

5. Conclusion

As we gain an improved understanding of the complexity of leukemia progression, the role of immunotherapy will become more important in the elimination of LSCs to cure leukemia. Furthermore, the use of immunotherapy to target LSCs in diverse types of leukemia, especially ALL, remains an important goal.

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